Variations in the *BDNF* Gene in Autopsy-Confirmed Alzheimer's Disease and Dementia with Lewy Bodies in Japan

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Key Words

Brain-derived neurotrophic factor · Alzheimer's disease · Parkinson's disease · Dementia with Lewy bodies · Single-nucleotide polymorphism

Abstract

Background/Aim: Brain-derived neurotrophic factor (BDNF) is associated with the hippocampus and the nigrostriatal dopaminergic function. Data showing that its level was reduced in Alzheimer's disease (AD) and Parkinson's disease (PD) suggested that the BDNF function must play an important role in the pathogenetics of these diseases. Indeed, variation in the *BDNF* gene may confer susceptibility to AD and PD development. Recently, a functional *BDNF* Val66Met polymorphism has been found to be associated with episodic memory and hippocampal function, with intracellular trafficking, and with activity-dependent secretion of BDNF. To date, there have been several conflicting reports on the correlation between AD or PD and Val66Met or C270T polymorphism in the *BDNF* promoter region, although no data on this relationship have been published with respect to dementia with Lewy bodies (DLB). In the present

study, we investigated a possible association between such *BDNF* polymorphisms and susceptibility to AD or DLB. **Methods:** *BDNF* genotyping was carried out by the polymerase chain reaction-restriction fragment length polymorphism method in autopsy-confirmed human samples. **Results and Conclusion:** On comparing patients and controls, the distribution of *BDNF* genotypes and alleles did not differ significantly. Our findings suggest that it is unlikely that these *BDNF* polymorphisms play a major role in the pathogenesis of AD and DLB in the Japanese population.

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Introduction

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein which is a member of the nerve growth factor family and is widely expressed in the adult mammalian brain, especially in the hippocampus [1]. It has been found to promote the survival of hippocampal and neocortical neurons. Moreover, the gene product has been involved in development, regeneration, and maintenance of neuronal systems [2]. A selective reduction in

the BDNF mRNA expression [3] as well as in the protein level [2, 4, 5] was first demonstrated in the hippocampal formation of Alzheimer's disease (AD) patients. In addition, a later study [6] showed that the proBDNF protein level is decreased in the parietal cortex of AD patients. These findings implicated BDNF in the pathogenesis of AD and suggested that the *BDNF* gene is a predisposing factor in AD development.

In a recent study performed in an Italian AD population [7], the authors investigated a functional single-nucleotide polymorphism (SNP; G196A) in the coding region of the *BDNF* gene which results in an amino acid change (Val66Met). In addition, Egan et al. [8] demonstrated that a SNP at G196A in the *BDNF* gene could affect intracellular processing and secretion of BDNF, leading to impairment in the hippocampal function. Moreover, these authors demonstrated the impact of this polymorphism on human brain function. In addition, Hariri et al. [9] showed that this SNP affects the human memory-related hippocampal activity and predicts the memory performance in healthy individuals. However, Nacmias et al. [10] analyzed the cognitive performance in AD patients with the *BDNF* Val66Met polymorphism and found it not to be an AD susceptibility factor, although these authors supported the effect of the apolipoprotein E (ApoE) ϵ 4 allele in increasing the risk of developing AD. On examining a potential role for the *BDNF* Val66Met polymorphism as an AD risk factor in Chinese [11] and Spanish [12] populations, the researchers found no association between this SNP and AD.

Furthermore, Kunugi et al. [13], who studied a group of Japanese AD patients, described another functional SNP (C-270T) in the promoter region of the *BDNF* gene. Subsequently, this allele was found to be frequently occurring in German AD patients [14] and a relevant risk factor for AD development. However, a group studying a Brazilian population did not obtain similar findings [15]. Finally, in another Italian study [16], these *BDNF* SNPs were rejected as having any association with AD. All of these patients had been diagnosed clinically as having probable AD. Therefore, with differential diagnoses, it is extremely important to distinguish dementia with Lewy bodies (DLB) from AD.

As for the relationship between BDNF and DLB, this factor has been strongly correlated with dopaminergic neurons [17], especially in patients having the disease. The substantia nigra of Parkinson's disease (PD) patients also showed reduced levels of BDNF mRNA [18] and protein as compared with control brains [19, 20]. Interestingly, one group [21] reported that glial BDNF was elevated

in the substantia nigra of PD patients. Three genes, *α -synuclein*, *parkin*, and *DJ-1*, have been clearly linked with familial PD [22], and, based on the findings mentioned above, it is reasonable to suggest that the *BDNF* gene may be linked with both PD and DLB pathogenesis. Momose and coworkers [23, 24] found that homozygous possession of the *BDNF* polymorphism, Val66Met, was associated with PD in Japanese patients as compared with unaffected controls. However, Swedish [25] and Chinese [26] groups were unable to replicate these findings in their own populations. The results of yet another Japanese group [27] were inconclusive. Although PD is a type of DLB, there have been no data as yet on the association between DLB and *BDNF* polymorphism.

All previous genetic analyses were based on clinical findings. By using neuropathologically diagnosed cases, we were able to clearly study the correlation between *BDNF* polymorphisms and major neurodegenerative dementias such as AD and DLB.

Patients and Methods

Patients

In the present study, the 267 cases examined consisted of patients hospitalized in the Fukushima Hospital. All of these patients were cognitively evaluated by neuropsychological testing, using such tests as the Mini-Mental State Examination [28] and Hasegawa's dementia scale [29] or its revised version [30] which is commonly utilized in Japan. We also recorded interviews employing a comprehensive questionnaire covering psychological and medical symptoms, chronic conditions, treatments, and activities of daily living. Autopsies were carried out at Fukushima Hospital, Japan, from October 1990, and *BDNF* genotyping was performed using DNA samples extracted from dissected brain tissues obtained between January 1993 and March 2003, after obtaining the agreement of the patients' guardians for use of these tissues for the purpose of diagnosis, research, and genetic analysis. This work was approved by the Ethical Committee of the Fukushima Hospital, February 30, 2004, and assigned application No. 177.

These samples are currently stored in the Fukushima Brain Bank. The patients consisted of 126 males and 141 females, with a mean age at the time of death of $82.7 \pm (\text{SD}) 8.49$ (range 44–105) years.

As a nondemented group, 108 elderly individuals were recruited from the Ehime University Graduate School of Medicine, and we have previously used this group as controls [31]. These population-based nondemented controls represented 87 females and 21 males with mean age at the time of blood drawing of $81.6 \pm (\text{SD}) 6.95$ years and an age range at the time of death of 70–101 years (table 1).

Autopsy and Sampling of Brain Tissues

The brain was removed at autopsy, weighed, cut midsagittally, and examined for vascular and other macroscopically detectable

Table 1. Summary of the main neuropathological subgroup diagnoses

	Males	Females	Total	Age at death years (mean $\pm$ SD)
Fukushima Brain				
Bank samples	126	141	267	82.7 $\pm$ 8.49
AD	37	58	95	83.5 $\pm$ 7.50
LNTD	2	2	4	95.0 $\pm$ 5.72
FTD	1	3	4	75.3 $\pm$ 4.50
DLB	16	18	34	81.3 $\pm$ 9.21
CVD	55	46	101	82.5 $\pm$ 8.37
Control brains	10	11	21	87.3 $\pm$ 6.70
Population based control samples	21	87	108	81.6 $\pm$ 6.95 ^a

AD = Alzheimer's disease; LNTD = limbic neurofibrillary tangle dementia; FTD = frontotemporal dementia; DLB = dementia with Lewy bodies; CVD = cerebrovascular disorders.

^a Age at the time blood was drawn.

lesions. Specimens for diagnostic examination were taken from the hemisphere showing abnormalities on CT scanning, or from the left hemisphere when no difference between the left and the right hemispheres was found, and fixed in 4% paraformaldehyde as a hemisphere block. The other hemisphere was divided into several regions. Some lesion samples were frozen for further analyses and stored at -80°C , while others were removed and fixed in 4% paraformaldehyde for immunohistochemical analysis.

Samples for diagnostic purposes were taken from several portions of the brain, as described previously [32]. The specimens were embedded in paraffin and processed into 5- μm sections for conventional histological and immunohistochemical examination.

Neuropathological Diagnostic Criteria

The specimens were stained using hematoxylin-eosin and Klüber-Barrera methods. Methenamine-silver staining was used to detect senile plaques, cerebral amyloid angiopathy, and neurofibrillary tangles (NFTs) [33]. Ubiquitin, α -synuclein, A β , and tau immunostaining methods were also used when necessary.

In addition to scoring according to CERAD criteria, senile plaques and NFTs as indicative of AD pathology were quantified as described by Mölsa et al. [34]. We have previously reported diagnostic criteria for limbic NFT dementia [32, 35–38] as well as other disease criteria which we have used previously [32].

Clinical neuropathological diagnoses of DLB and subtype differentiation were based on DLB guidelines [39] and on the findings described in other reports [32, 40, 41]. Among our control brains, there were no pathological findings other than the physiological changes of aging.

BDNF Subtyping

DNA of the autopsied cases was extracted from brain tissues by the phenol-chloroform method. The peripheral blood from the pop-

ulation-based nondemented group of elderly subjects was collected into tubes containing EDTA, and DNA was extracted using a QIAamp DNA Blood Mini kit (Qiagen, Valencia, Calif., USA) and stored at 4°C . *BDNF* genotyping was carried out by means of the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method, according to a procedure used by Ventriglia et al. [7] to analyze Val66Met and by Kunugi et al. [13] to analyze C270T. As for the analysis of ApoE in AD and DLB patients, we have described the methods used and the results obtained in a previous report [32].

Statistics

Statistical analysis was carried out with both the χ^2 test with Yates' correction and Fisher's exact test using 2×2 tables. A difference was considered significant, if $p < 0.05$ and the odds ratio had a 95% confidence interval (CI).

Results

Frequencies of Neuropathological Findings

Frequencies and mean ages at the time of death of the neuropathologically diagnosed subgroups are summarized in table 1. The main neuropathological findings of the Brain Bank samples were cerebrovascular disorders (cerebral infarcts and hemorrhages with or without dementia; 38%), AD (36%), and DLB (13%). Two or three types of diagnostic changes were used as criteria for more than one disease. Percentages of each of the main neuropathological diagnoses were similar to those described in our previous report [35]. The age distribution of the Brain Bank and population-based samples was similar.

Frequencies of *BDNF* Alleles and Genotypes in the Main Neuropathological Disorders

Since only 21 (8%) of the Fukushima Brain Bank samples showed signs of physiological aging alone, we used the population-based nondemented group of elderly subjects as a control in comparing alleles and genotype frequencies of the *BDNF* gene (table 2a). The genotype distribution for any patient or control group did not deviate significantly from the Hardy-Weinberg equilibrium, excluding Val66Met in the AD group. We do not know whether the Val66Met heterozygotes in AD exceeded the theoretical Hardy-Weinberg equilibrium ratio. Along with this population-based nondemented group of elderly subjects, groups representative of the main neurological diseases, AD, limbic NFT dementia, frontotemporal dementia, DLB, and cerebrovascular disorders, were analyzed for C270T and Val66Met polymorphisms of the *BDNF* gene. Our data show that no group had TT at

Table 2a. Association between C270T and Val66Met polymorphisms of the *BDNF* gene and neuropathological findings compared with those of the population-based control group

	AD	LNTD	FTD	DLB	CVD	Control brains	Popula- tion-based controls
C270T							
CC (%)	89 (94)	3 (75)	4 (100)	31 (91)	98 (97)	20 (95)	101 (94)
CT (%)	6 (6)	1 (25)	0 (0)	3 (9)	3 (3)	1 (5)	7 (6)
TT (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele C (%)	184 (97)	7 (88)	8 (100)	65 (96)	199 (99)	41 (98)	209 (97)
Allele T (%)	6 (3)	1 (12)	0 (0)	3 (4)	3 (1)	1 (2)	7 (3)
Odds ratio (95% CI)	1.03 (0.34–3.11)	4.26 (0.46–39.53)	–	1.38 (0.35–5.48)	2.22 (0.18–2.89)	/	reference
Val66Met							
Val/Val (%)	25 (26)	1 (25)	1 (25)	12 (35)	28 (28)	6 (29)	35 (32)
Val/Met (%)	58 (61)	3 (75)	1 (25)	17 (50)	55 (54)	10 (48)	53 (49)
Met/Met (%)	12 (13)	0 (0)	2 (50)	5 (15)	18 (18)	5 (23)	20 (19)
Allele Val (%)	108 (57)	5 (63)	3 (37)	41 (60)	111 (55)	22 (52)	123 (57)
Allele Met (%)	82 (43)	3 (37)	5 (63)	27 (40)	91 (45)	20 (48)	93 (43)
Odds ratio (95% CI)	1.00 (0.67–1.48)	1.26 (0.29–5.41)	2.20 (0.51–9.46)	1.15 (0.66–2.00)	1.08 (0.74–1.60)	/	reference

For explanation of abbreviations see table 1.

As compared with population-based controls, no group showed a statistically significant difference ($p < 0.05$, odds ratio with a 95% CI which included 1.0).

Table 2b. Genotype distribution of *BDNF* C270T and Val66Met polymorphisms in patients with DLB subtypes

	DLB total	Brain stem type	Transient type	Neocortical type	Popula- tion-based controls
C270					
CC (%)	34	10	14	10	108
CT (%)	31 (91)	9 (90)	12 (86)	10 (100)	101 (94)
TT (%)	3 (9)	1 (10)	2 (14)	0 (0)	7 (6)
Allele C (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele T (%)	65 (96)	19 (95)	26 (93)	20 (100)	209 (97)
Odds ratio (95% CI)	3 (4)	1 (5)	2 (7)	0 (0)	7 (3)
	1.38 (0.35–5.48)	1.57 (0.18–13.45)	2.30 (0.45–11.65)	–	reference
Val66Met					
Val/Val (%)	12 (35)	4 (40)	5 (36)	3 (30)	35 (32)
Val/Met (%)	17 (50)	5 (50)	7 (50)	5 (50)	53 (49)
Met/Met (%)	5 (15)	1 (10)	2 (14)	2 (20)	20 (19)
Allele Val (%)	41 (60)	13 (65)	17 (61)	11 (55)	123 (57)
Allele Met (%)	27 (40)	7 (35)	11 (39)	9 (45)	93 (43)
Odds ratio (95% CI)	1.45 (0.66–2.00)	1.40 (0.54–3.66)	1.17 (0.52–2.61)	1.08 (0.43–2.72)	reference

As compared with population-based controls, no group showed a statistically significant difference ($p < 0.05$, odds ratio with a 95% CI which included 1.0).

C270T (table 2a). For Val66Met, three PCR-FRLP patterns emerged (data not shown). Val/Met comprised around 50% of each group, except for the frontotemporal dementia group, and the distribution pattern was similar

to that of the population-based controls. When comparing each main neurological disease group with the findings in the population-based controls, the odds ratio had a 95% CI which included 1.0 (table 2a).

Table 3. Analysis of BDNF SNP patterns with ApoE ϵ 4 status and age at AD and DLB onset

ApoE ε4	AD		DLB		Popula- tion-based controls
	+	–	+	–	
C270T					
CC (%)	45 (92)	45 (96)	11 (100)	20 (87)	101 (94)
CT (%)	4 (8)	2 (4)	0 (0)	3 (13)	7 (6)
TT (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele C (%)	94 (96)	92 (98)	22 (100)	43 (93)	209 (97)
Allele T (%)	4 (4)	2 (2)	0 (0)	3 (7)	7 (3)
Odds ratio (95% CI)	1.27 (0.36–4.44)	1.54 (0.31–7.56)	–	2.08 (0.52–8.38)	reference
Val66Met					
Val/Val (%)	12 (24)	13 (28)	3 (27)	9 (39)	35 (32)
Val/Met (%)	29 (59)	30 (64)	5 (46)	12 (52)	53 (49)
Met/Met (%)	8 (17)	4 (8)	3 (27)	2 (9)	20 (19)
Allele Val (%)	53 (54)	56 (60)	11 (50)	30 (65)	123 (57)
Allele Met (%)	45 (46)	38 (40)	11 (50)	16 (35)	93 (43)
Odds ratio (95% CI)	1.12 (0.69–1.81)	1.11 (0.68–1.82)	1.32 (0.55–3.18)	1.42 (0.73–2.75)	reference

As compared with the population-based controls, no group showed a statistically significant difference ($p < 0.05$, odds ratio with a 95% CI which included 1.0).

Frequencies of BDNF Alleles and Genotypes in DLB

The DLB group as a whole was not significantly different from the population-based controls with respect to C270T and Val66Met. In a previous report, harboring a Val66Met polymorphism in the *BDNF* gene was described as a genetic risk factor for PD, as in the brain stem type of DLB. Our 34 DLB cases were classified according to their subclass following DLB guidelines [39], supported by information gathered in previous studies [32, 40, 41]. No subgroup showed any remarkable odds ratio inclination (table 2b).

Analysis of BDNF SNP Patterns with ApoE ϵ 4 Status and Age at AD and DLB Onset

From the above, we conclude that having a C270T or Val66Met polymorphism in the *BDNF* gene or in a gene nearby represents a relevant genetic risk factor for developing AD or DLB, particularly in patients with lacking the ApoE ϵ 4 allele, as determined using our Japanese samples. For our AD and DLB patients, we determined whether the ApoE ϵ 4 status was positive or negative, but, as shown in table 3, we could not detect any statistical correlation with the *BDNF* polymorphisms. AD/DLB samples with or without the ApoE ϵ 4 allele were compared with samples from population-based controls using a mean odds ratio (95% CI which included 1.0).

Discussion

BDNF appears to be a trophic factor for mesencephalic dopaminergic neurons and increases their survival, including that of neuronal cells which degenerate in PD [17]. This factor provides for long-term neuronal adaptation by controlling the responsiveness of its target neurons to the important neurotransmitter, dopamine. BDNF has been shown to control dopamine D3 receptor expression and to trigger behavioral sensitization [42]. BDNF also modulates hippocampal plasticity and hippocampus-dependent memory in cell models and in animals [8]. Moreover, the decrease in the transcript level of BDNF mRNA in hippocampi of individuals with AD was verified with an RNAase protection assay, suggesting that BDNF may contribute to the progression of cell death in AD patients [3].

A report appearing in 2001 [13] showed that C270T functional promoter polymorphism in the *BDNF* gene represented an AD risk factor. In 2002, other groups [7, 23] reported independently that an SNP at Val66Met in BDNF raises the likelihood of AD [7] or PD [23]. Moreover, a functional analysis demonstrated a role for BDNF and its Val66Met polymorphism in human episodic memory and hippocampal function and suggested that Val66Met exerts these effects by impacting intracellular trafficking and activity-dependent secretion of BDNF [8].

After this report, several *BDNF* SNP analyses appeared, as mentioned in the Introduction. These SNPs were generally considered risk factors for both diseases, but recent reports have not mentioned this possibility. According to Shimizu et al [43], the rates of *BDNF* SNP depend on ethnic differences. Our incidence of Val66Met was almost the same as that found by these authors which was also estimated from Japanese data. In addition, Desai et al. [44] recently described a difference in Val66Met and C270T distribution patterns of Caucasian Americans and African-Americans [44]. In case of C270T polymorphism, our results were similar to those reported by another Japanese group [13] as well as to the findings observed in African-Americans [44].

As for the risk of sporadic DLB, we reported that ApoE is a predisposing factor for this disease as it is for AD [32]. Moreover, paraoxonase-1 [45] is associated with the Lewy body stage [45]. However, harboring a *BDNF* polymorphism has not conclusively been shown to raise the PD risk, while no data are available on DLB.

The aim of our current study was to determine whether the Val66Met and C270T polymorphisms represent risk factors for developing AD or DLB, and for this task we used autopsy samples, with which the diagnosis had been confirmed. *BDNF* has been found to promote survival of all major neuronal tissue types affected in AD and PD/DLB, such as hippocampal and neocortical neurons, cholinergic septal and basal forebrain neurons, and nigral dopaminergic neurons [2]. Taken together, these findings indicate that *BDNF* plays a pivotal role in protecting hippocampal and nigral neurons, and evidence of its dysfunction could be suggestive of AD or DLB pathogenesis.

Among our neuropathologically diagnosed samples, no subgroup showed an association with *BDNF* SNPs (table 2a). *BDNF* SNPs might raise the risk of AD, but our data could not confirm this. As for DLB, none of the DLB subgroups showed any relationship to *BDNF* polymorphisms C270T and Val66Met. Our DLB samples were taken from a total of 35 patients, and the size of this group might have been too small to establish statistical significance.

It is known that ApoE polymorphism is a strong risk factor for AD and DLB [32]. With this in mind, we filtered our data according to the ApoE ϵ 4+/- status (table 3), but we failed to detect any correlation.

In conclusion, variation in the C270T rate was too great to provide a useful measure of the genetic risk for a particular individual, and as for the Val66Met SNP, no correlations were observed with our Japanese AD and DLB patients. In assessing the biological function of *BDNF* in AD, DLB, and PD, we could not detect any association with *BDNF* SNPs. To reach at a final conclusion, the next step must involve the study of the genetic background of individual patients with a sufficient number of samples, for which the neuropathological diagnosis has been confirmed.

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